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EXAMINER	
LU, FRANK WEI MIN	

ART UNIT	PAPER NUMBER
1634	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/796,298	MITSUHASHI, MASATO	
	Examiner	Art Unit	
	FRANK W. LU	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 April 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-38,73-75,77-93 and 215-219* is/are pending in the application.

4a) Of the above claim(s) *13,14,16-26,30-33,89 and 90* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-12,15,27-29,34-38,73-75,77-88,92,93 and 215-219* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *2/13/2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

1. Applicant's response to the office action filed on December 17, 2007 and applicant's response to the office communication filed on April 18, 2008 have been entered. The claims pending in this application are claims 1, 3-38, 73-75, 77-93, and 215-219 wherein claims 13, 14, 16-26, 30-33, 89, and 90 have been withdrawn due to species election mailed on September 28, 2006. Rejection and /or objection not reiterated from the previous office action are hereby withdrawn in view of applicant's amendment filed on April 18, 2008. Claims 1, 3-12, 15, 27-29, 34-38, 73-75, 77-88, 92-93, and 215-219 will be examined.

Specification

2. The disclosure is objected to because of the following informality: although applicant amended claim 6 and indicated that the abbreviation PBT is polybutylene terephthalate, since there is no phrase "polybutylene terephthalate" in the specification, applicant is required to add this phrase into the specification.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. New Matter

Claims 27, 29, 34, 35, 73-75, 77-88, 91-93, and 215-219 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

First, the recitation "the specific mRNA is mRNA known to be induced during apoptosis development in leukemia" is added to newly amended dependent claim 27. Although the specification describes that the mRNA of apoptosis genes involved in leukemia is quantified (see original filed claim 27), the specification fails to define or provide any disclosure to support such claim recitation in claim 27 because the mRNA of apoptosis genes involved in leukemia recited in original filed claim 27 is not equal to and is broader than mRNA known to be induced during apoptosis development in leukemia. Second, the recitation "test the side effects of anti-cancer drugs that induce specific mRNA responsible for apoptosis development in leukocytes" is added to newly amended dependent claim 29. Although the specification describes to test the side effects of anti-cancer drugs on white blood cells (see original filed claim 29) and describes that the genes related to apoptosis are candidate genes for anti-leukemia drugs (see Table 1 in page 8), the specification fails to define or provide any disclosure to support such claim recitation in claim 29 because anti-cancer drugs recited in claim 29 is not limited to anti-leukemia drugs. Third, the recitation "the whole blood is exposed to donor cells prior to filtration" is added to the newly amended dependent claim 34 while the recitation "the quantification of a higher than normal level of the mRNA is indicative of transplant rejection" is added to the newly amended dependent claim 35. Although the specification describes that the mRNA of donor cell-mediated

cytokines is quantified wherein the quantification of mRNA of donor cell-mediated cytokines is used to test transplant rejection (see original filed claims 34 and 35), the specification fails to define or provide any disclosure to support such claim recitations in claims 34 and 35. Fourth, the recitation "determining the definite quantity of mRNA by dividing the value of sample mRNA determined in step (f) by applying the percent recovery of spiked control RNA determined in step (g)" is added to the newly amended dependent claim 73. Although the specification describes that a definite quantity of target mRNA can be determined by dividing the values obtained by the TaqMan assay with percent recovery of a dose of spiked control RNA in each sample (see page 14, second paragraph and page 28, first paragraph), the specification fails to define or provide any disclosure to support such claim recitations in claim 73. Fifth, although the specification describes a method of synthesizing cDNA in solution upon poly-A RNA, comprising application of specific antisense primers during hybridization of RNA poly-A tails and immobilized oligo(dT) and a method of synthesizing cDNA in solution upon poly-A RNA, comprising application of specific antisense primers during cDNA synthesis (see original filed claims 94 and 95), paragraph [0080] of the specification suggested by applicant does not describe the phrase "the cDNA formed by extension of the antisense primers is in solution" as recited in step (g) of claim 215 because this phrase is read as that the cDNA formed by extension of the antisense primers goes directly into solution. The specification also does not describe the cDNA formed by extension of the antisense primers goes into solution without heat denaturation as recited in claim 216 and a plurality of different antisense primers for different specific mRNAs are present in the lysis buffer as recited in claim 217. Note that paragraph [0080] of the specification suggested by applicant requires that the cDNA formed by extension of oligo(dT)

displaces the cDNA formed by extension of the antisense primer during amplification so that the cDNA formed by extension of the antisense primer goes into solution without heat denaturation (ie., the heat denaturing process is completely eliminated). Sixth, in applicant's remarks filed on April 18, 2008, applicant does not indicate which parts in the specification supports such claim recitations recited in claims 29, 34, 35, and 73.

MPEP 2163.06 notes "IF NEW MATTER IS ADDED TO THE CLAIMS, THE EXAMINER SHOULD REJECT THE CLAIMS UNDER 35 U.S.C. 112, FIRST PARAGRAPH - WRITTEN DESCRIPTION REQUIREMENT. *IN RE RASMUSSEN*, 650 F.2D 1212, 211 USPQ 323 (CCPA 1981)." MPEP 2163.02 teaches that "Whenever the issue arises, the fundamental factual inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed...If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application." MPEP 2163.06 further notes "WHEN AN AMENDMENT IS FILED IN REPLY TO AN OBJECTION OR REJECTION BASED ON 35 U.S.C. 112, FIRST PARAGRAPH, A STUDY OF THE ENTIRE APPLICATION IS OFTEN NECESSARY TO DETERMINE WHETHER OR NOT "NEW MATTER" IS INVOLVED. *APPLICANT SHOULD THEREFORE SPECIFICALLY POINT OUT THE SUPPORT FOR ANY AMENDMENTS MADE TO THE DISCLOSURE*" (emphasis added).

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 28, 29, 35, 73-75, 77-88, and 91-93 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

7. Claim 28 is rejected as vague and indefinite. Since claims 1 and 29 do not indicate that the specific mRNA is the mRNA of cytokines, it is unclear why the mRNA of cytokines can be quantified. Please clarify.

8. Claim 29 is rejected as vague and indefinite. Since claims 1 and 29 do not indicate that the specific mRNA is a specific mRNA responsible for apoptosis development in leukocytes, it is unclear how the quantification of mRNA is used to test the side effects of anti-cancer drugs that induce specific mRNA responsible for apoptosis development in leukocytes. Please clarify.

9. Claim 35 is rejected as vague and indefinite. Since claims 1 and 35 do not indicate that the specific mRNA is related to transplant rejection, it is unclear why the quantification of a higher than normal level of the mRNA can be indicative of transplant rejection. Please clarify.

10. Claim 73 recites the limitation "dividing the value of spiked control RNA determined in step (f) by the amount of spiked control RNA obtained in step (d)" in step (g) of the claim. There is insufficient antecedent basis for this limitation in the claim because there are no value of spiked control RNA in step (f) and no amount of spiked control RNA in step (d). Please clarify.

11. Claim 73 recites the limitation "dividing the value of sample mRNA determined in step (f)" in step (h) of the claim. There is insufficient antecedent basis for this limitation in the claim because there is no value of sample mRNA in step (f). Please clarify.

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 1, 3, 5, 11, 12, 15, and 36-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ishikawa *et al.,* (Clinical Chemistry, 43, 764-770, 1997) in view of Mitsuhashi

(WO 99/32654, published on July 1, 1999) and Garvin (US 2003/0170669 A1, priority date: April 11, 2000).

Regarding claims 1 and 3, Ishikawa *et al.,* teach collecting whole blood, administering an anticoagulant (ie., heparin) to the whole blood, removing erythrocytes and blood components other than leukocytes from the whole blood to yield leukocytes (ie., by centrifugation), lysing the leukocytes to produce a lysate comprising mRNA including said specific mRNA, transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA, and quantifying the specific mRNA (ie., by quantifying synthesized cDNA) as recited in steps (a) to (f) of claim 1 where heparin is administered to the whole blood prior to collection of leukocytes as recited in claim 3 (see page 765, right column, page 766, left column and last paragraph of right column).

Regarding claim 11, Ishikawa *et al.,* teach that the immobilized plate comprises a multi-well oligo(dT)-immobilized plate (see page 765, right column, last paragraph and page 766, left column, first paragraph).

Regarding claim 15, Ishikawa *et al.,* teach the quantification of mRNA comprises cDNA synthesis of the specific mRNA (ie., ODC mRNA) and amplification of resulting cDNA (see page 766).

Regarding claim 36, Ishikawa *et al.,* teach additionally comprising determining the quantity of target mRNA in the sample using spiked control RNA (ie., rabbit globin mRNA) (see page 766).

Regarding claim 37, Ishikawa *et al.,* teach additionally comprising application of specific antisense primers during said lysate transferring step (ie., hybridizing oligo(dT) on the GenePlate

to the mRNA in said lysate wherein oligo(dT) can serve as a primer) (see page 765, right column, last paragraph and page 766, left column, first paragraph).

Regarding claim 38, Ishikawa *et al.,* teach additionally comprising application of specific antisense primers (ie., antisense primer in PCR reaction) during said mRNA quantification step (see page 766).

Ishikawa *et al.,* do not disclose removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes comprising eosinophils on a filter membrane and lysing the leukocytes on a filter membrane as recited in steps (c) and (d) of claim 1 wherein the filter membrane is attached to a multi-well filter plate as recited in claim 5, that the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation as recited in claim 12. However, Ishikawa *et al.,* teach removing erythrocytes and blood components other than leukocytes from the whole blood by centrifugation to yield leukocytes and lysing the leukocytes in a lysis buffer (see page 765, right column).

Since it is known that leukocytes contain 1-6% eosinophils (see attached definition of white blood cell from Wikipedia, the free encyclopedia), Mitsuhashi teach yielding leukocytes comprising eosinophils on a filter membrane and lysing the leukocytes on a filter membrane as recited in steps (c) and (d) of claim 1 wherein the filter membrane is attached to a multi-well filter plate as recited in claim 5 and that the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation as recited in claim 12 (see pages 7 and 8).

Garvin teaches removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane as recited in step (c) of claim 1 (see page 1).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to have performed the methods recited in claims 1 and 5 by removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes comprising eosinophils on a filter membrane and lysing the leukocytes on a filter membrane wherein the filter membrane is attached to a multi-well filter plate in view of the prior art of Ishikawa *et al.,* Mitsuhashi, and Garvin. One having ordinary skill in the art would have been motivated to do so because Mitsuhashi suggests that "[B]y placing cells on a filter membrane evenly and passing a lysis buffer through the cell layer on the filter membrane without mechanical homogenization of the cells, it is possible to drastically simplify the preparation of cell lysate and significantly stabilize the yield of recovered cytosolic RNA" (see page 6, third paragraph) and the simple substitution of one kind of filter (ie., the filter such as glass fiber taught by Mitsuhashi) from another kind of filter (ie., the filter such as Leukotrap WB taught by Garvin) for removing erythrocytes and blood components other than leukocytes from the whole blood during the process for performing the methods recited in claims 1 and 5, in the absence of convincing evidence to the contrary, would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made since the filter such as Leukotrap WB taught by Garvin is commercially available and has an ability to remove more than 99.9% of leucocytes from one unit of while blood (see page 1, [0006] and [0007]).

Furthermore, the motivation to make the substitution cited above arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making the obviousness rejection comes from the M.P.E.P. at 2144.06, 2144.07 and 2144.09.

Also note that there is no invention involved in combining old elements is such a manner that these elements perform in combination the same function as set forth in the prior art without giving unobvious or unexpected results. *In re Rose* 220 F.2d. 459, 105 USPQ 237 (CCPA 1955).

Response to Arguments

In page 9, seventh paragraph bridging to page 11, last paragraph of applicant's remarks, applicant argues that: (1) "[A]pplicant's use of filtration to obtain leukocytes results in collection of most or all subsets of leukocytes, including eosinophils. Eosinophils contain a large quantity of RNases. By contrast, the centrifugation method employed by Ishikawa *et al.* uses a density gradient to collect mononuclear leukocytes, in which the RNase content is very small. One skilled in the art would not have any reason to retain the RNase-rich leukocytes when conducting an assay for mRNA, as the RNases present in leukocytes would be expected to degrade the mRNA being assayed. As a result, it would be counter-intuitive for one skilled in the art to combine the teachings of Ishikawa *et al.* with those of Mitsuhashi and/or Garvin. However, applicants have unexpectedly discovered that the degradation of the mRNA can be avoided by lysing the leukocytes with an appropriate lysis buffer directly on the filter membrane to produce a lysate that contains the mRNA. Because it is only Applicant's discovery that would lead to a combination of the references cited by the Examiner, the cited references do not create a *prima facie* showing of obviousness"; (2) "even if one having ordinary skill in the art were to combine Ishikawa *et al.* with Mitsuhashi and Garvin in the manner suggested by the Examiner, none of these references would lead such a person to predict the significant unexpected results obtained by the combination. The Examples in the specification directly compare the results obtained using the claimed method with that of conventional density gradient separation of peripheral

blood mononuclear cells (PBMC) as described in Ishikawa *et al.* For example, in Example 7, the disclosed filtration method results in significantly improved recovery of mRNA compared to conventional density gradient and centrifugation separation methods like that used by Ishikawa *et al. See* Spec., Paragraph [0134]. The disclosed filtration method also results in superior uniformity of mRNA recovery. *See* Spec., Paragraph [0135]. In addition, a wide range of lysis buffer concentrations are suitable for optimum performance, demonstrating reproducibility and robustness. *See* Spec., Paragraph [0137]"; (3) "as shown in both Examples 5 and 7, the total assay efficiency is sequence-independent. *See* Spec., Paragraphs [0125] and [0143]. On account of these significant unexpected results and in light of the long-felt need in the field for a high-throughput method of quantifying mRNA, Ishikawa *et al.* taken in view of Mitsuhashi and Garvin does not render Claim 1 obvious. These unexpected results are strong evidence of nonobviousness that would rebut *a prima facie* showing of obviousness even were such a showing present"; (4) "[C]laim 36 recites the additional limitation of determining the quantity of target mRNA in the sample using spiked control RNA. The recovery of control RNA should be the same for various target mRNAs for the control RNA in question to serve as a universally applicable control RNA. However, the recovery of control RNA may vary depending on the length, sequence, and abundance of mRNA. Thus, prior to Applicant's invention, no consensus was previously available whether development of a universal control RNA was even possible. Thus, Claim 36, which recites the use of such spiked control RNA, is nonobvious in view of the disclosure of the cited references for this additional reason as well"; and (5) "[T]he cited Ishikawa *et al.* reference discloses the use of an oligo(dT) primer which is immobilized on a solid support. Any mRNA that is bound to the oligo(dT) can be extended using the oligo(dT) as a

primer. Thus all of the primed cDNA that is generated in Ishikawa *et al.* is immobilized on a solid support until it is denatured. By contrast, through the addition of specific antisense primers, Applicant achieves release of cDNA into the solution phase without the necessity of heat denaturation. As described in Paragraph [0080] and shown in Figure 15, cDNA is primed by both the oligo(dT) and by the antisense primers. The cDNA derived from extension of the antisense primers is displaced by cDNA derived from extension of the bound oligo(dT). Thus, the cDNA derived from extension of the antisense primers can enter the solution phase following displacement by the oligo(dT)-derived cDNA during amplification. Remarkably, this cDNA displacement occurs without heat denaturation. Nothing in the Ishikawa *et al.* reference, or any of the other references cited by the Examiner, discloses this feature".

These arguments have been fully considered but they are not persuasive toward the withdrawal of the rejection. First, although applicant argues that "[O]ne skilled in the art would not have any reason to retain the RNase-rich leukocytes when conducting an assay for mRNA, as the RNases present in leukocytes would be expected to degrade the mRNA being assayed", applicant has no evidence to show that one skilled in the art must exclude eosinophilis from leukocytes before he or she isolates mRNA from leukocytes in order to exclude RNase-rich leukocytes. Furthermore, since it is routine to add RNase inhibitors during the process of RNA isolation, RNases released from cells can be inhibited. Note that RNA isolated from leukocytes excluding eosinophilis is not considered as total from leukocyte RNA. Second, in view of the prior art of Ishikawa *et al.,* Mitsuhashi, and Garvin, removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes comprising eosinophilis on a filter membrane and lysing the leukocytes on a filter membrane

wherein the filter membrane is attached to a multi-well filter plate as recited in claim 1 are expected because Mitsuhashi suggests that "[B]y placing cells on a filter membrane evenly and passing a lysis buffer through the cell layer on the filter membrane without mechanical homogenization of the cells, it is possible to drastically simplify the preparation of cell lysate and significantly stabilize the yield of recovered cytosolic RNA" (see page 6, third paragraph) and the simple substitution of one kind of filter (ie., the filter such as glass fiber taught by Mitsuhashi) from another kind of filter (ie., the filter such as Leukotrap WB taught by Garvin) for removing erythrocytes and blood components other than leukocytes from the whole blood during the process for performing the methods recited in claims 1 and 5, in the absence of convincing evidence to the contrary, would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made since the filter such as Leukotrap WB taught by Garvin is commercially available and has an ability to remove more than 99.9% of leucocytes from one unit of while blood (see page 1, [0006] and [0007]). Third, although applicant argues that "in Example 7, the disclosed filtration method results in significantly improved recovery of mRNA compared to conventional density gradient and centrifugation separation methods like that used by Ishikawa *et al. See* Spec., Paragraph [0134]. The disclosed filtration method also results in superior uniformity of mRNA recovery. *See* Spec., Paragraph [0135]", the paragraphs [134] and [135] only describes that filterplate's hybridization performances of standard RNA, CD4, p21 (A), FasL, and leukotrien C4 synthese mRNA (LTC4S) are slightly better than that of the density gradient method. Fourth, claim 36 does not require that the recovery of control RNA should be the same for various target mRNAs for the control RNA in question to serve as a universally applicable control RNA as argued by applicant.

Fifth, claim 37 does not require release of cDNA into the solution phase without the necessity of heat denaturation and does not require that the cDNA derived from extension of the antisense primers is displaced by cDNA derived from extension of the bound oligo(dT) and the cDNA derived from extension of the antisense primers enter the solution phase following displacement by the oligo(dT)-derived cDNA during amplification as argued by applicant.

14. Claim 4 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ishikawa *et al.,* Mitsuhashi and Garvin as applied to claims 1, 3, 5, 11, 12, 15, and 36-38 above, and further in view of Augello *et al.,* (US Patent No. 6,617,170 B2, filed on October 30, 2001).

The teachings of Ishikawa *et al.,* Mitsuhashi and Garvin have been summarized previously, *supra.*

Ishikawa *et al.,* Mitsuhashi and Garvin do not disclose that the whole blood is frozen and subsequently thawed prior to filtration as recited in claim 4.

Augello *et al.,* teach to isolate RNA using frozen and subsequently thawed whole blood (see column 9, second paragraph).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to have performed the method recited in claim 4 wherein in view of the whole blood is frozen and subsequently thawed prior to filtration in view of the prior art of Ishikawa *et al.,* Mitsuhashi, Garvin, and Augello *et al.*. One having ordinary skill in the art would have been motivated to do so because isolating RNA using frozen and subsequently thawed whole blood would let one having ordinary skill in the art to use collected whole blood for multiple time and save time and cost. One having ordinary skill in the art at the time the

invention was made would have a reasonable expectation of success to perform the method recited in claim 4 using frozen and subsequently thawed whole blood.

15. Claims 6 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ishikawa *et al.,* Mitsuhashi and Garvin as applied to claims 1, 3, 5, 11, 12, 15, and 36-38 above, and further in view of Pall (US Patent No. 4,923,620, published on May 8, 1990).

The teachings of Ishikawa *et al.,* Mitsuhashi and Garvin have been summarized previously, *supra*.

Ishikawa *et al.,* Mitsuhashi and Garvin do not disclose that the filter membrane is a PBT fibrous membrane as recited in claim 6 wherein the leukocytes are captured on a plurality of filter membranes layered together as recited in claim 7.

Pall teaches to use polyester PBT for leukocyte depletion from blood (see abstract, column 19, lines 40-67, column 20, lines 1-20, and column 27, lines 22-28) and combine two, three or more layers of fiber to form an integral element for adsorption of a portion of the leukocytes (see column 22, lines 1-24).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to have performed the methods recited in claims 6 and 7 wherein the filter membrane is a PBT fibrous membrane and the leukocytes are captured on a plurality of filter membranes layered together in view of the prior art of Ishikawa *et al.,* Mitsuhashi, Garvin, and Pall. One having ordinary skill in the art would have been motivated to do so because comparing with other leukocyte depletion resins, polyester PBT is a preferred material since "it also lends itself to radiation grafting and to subsequent conversion into

preformed elements of controlled pore size by hot pressing" (see Pall, column 19, lines 63-67 and column 20, lines 1-4) and the integral element formed by two, three or more layers of fiber would provide large portion of the fiber surface on which leukocytes are removed by adsorption (see Pall, column 22, lines 1-24), and the simple substitution of one kind of filter (ie., the filter such as Leukotrap WB taught by Garvin) from another kind of filter (ie., PBT filter taught by Pall) for removing erythrocytes and blood components other than leukocytes from the whole blood during the process for performing the methods recited in claims 6 and 7, in the absence of convincing evidence to the contrary, would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made.

Furthermore, the motivation to make the substitution cited above arises from the expectation that the prior art elements will perform their expected functions to achieve their expected results when combined for their common known purpose. Support for making the obviousness rejection comes from the M.P.E.P. at 2144.07 and 2144.09.

Also note that there is no invention involved in combining old elements is such a manner that these elements perform in combination the same function as set forth in the prior art without giving unobvious or unexpected results. *In re Rose* 220 F.2d. 459, 105 USPQ 237 (CCPA 1955).

Response to Arguments

In page 12, first paragraph of applicant's remarks, applicant argues that "[T]he Examiner rejected Claims 6 and 7 as unpatentable over Ishikawa *et al.* in view of Mitsuhashi and Garvin and in further view of Pall. The foregoing amendments to Claim 1 should automatically render Claims 6 and 7 in condition for allowance based upon their dependence upon Claim 1".

This argument has been fully considered but it are not persuasive toward the withdrawal of the rejection because the combination of Ishikawa *et al.,* Mitsuhashi, and Garvin does teach all limitations of claims 1 and 5 (see above Response to Arguments related to item No. 13) and the combination of Ishikawa *et al.,* Mitsuhashi, Garvin, and Pall does teach all limitations of claims 6 and 7.

16. Claims 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ishikawa *et al.,* Mitsuhashi and Garvin as applied to claims 1, 3, 5, 11, 12, 15, and 36-38 above, and further in view of Naef (US Patent No. 5,177,085, published on January 5, 1993).

The teachings of Ishikawa *et al.,* Mitsuhashi and Garvin have been summarized previously, *supra.*

Ishikawa *et al.,* Mitsuhashi and Garvin do not disclose washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components as recited in claim 8. Since Mitsuhashi teaches to vacuum aspirate the filter plate to trap cells onto membranes (see page 7, step 1), Mitsuhashi discloses drying the filter membrane as recited in claim 9.

Naef teaches to remove erythrocytes by hypotonic lysis (see column 8, third paragraph).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to have performed the method recited in claim 8 by washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components in view of the prior art of Ishikawa *et al.,* Mitsuhashi, Garvin, and Naef. One having ordinary skill in the art would have been motivated to do so because the addition of

hypotonic lysis into whole blood would remove erythrocytes from whole blood (see Naef, column 8, third paragraph). One having ordinary skill in the art at the time the invention was made would have been a reasonable expectation of success to wash the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components in view of the prior art of Ishikawa *et al.,* Mitsuhashi, Garvin, and Naef.

Response to Arguments

In page 12, second paragraph of applicant's remarks, applicant argues that "[T]he Examiner rejected Claims 8 and 9 as unpatentable over Ishikawa *et al.* in view of Mitsuhashi and Garvin and in further view of Naef. The foregoing amendments to Claim 1 should automatically render Claims 8 and 9 in condition for allowance based upon their dependence upon Claim 1. However, in light of the Examiner's remarks Applicant wishes to provide additional reasons why Claim 8 is nonobvious. Leukocyte preparation using a hypotonic solution wash coupled with a centrifugation method of collecting leukocytes reduces the recovery of mRNA. This may be because some leukocytes are damaged, resulting in mRNA leakage during the centrifugation step. However, employing a hypotonic solution wash with a filtration method of collecting leukocytes does not result in an analogous reduction in mRNA recovery. Thus, even though washing with a hypotonic solution was known, employing a hypotonic solution wash on a filter is unique and nonobvious".

These arguments have been fully considered but they are not persuasive toward the withdrawal of the rejection. First, the combination of Ishikawa *et al.,* Mitsuhashi, and Garvin does teach all limitations of claims 1 and 5 (see above Response to Arguments related to item No. 13). Second, the office action does not indicate to couple Leukocyte preparation using a

hypotonic solution wash with a centrifugation method of collecting leukocytes as argued by applicant.

17. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ishikawa *et al.,* Mitsuhashi, Garvin and Naef as applied to claims 1, 3, 5, 8, 9, 11, 12, 15, and 36-38 above, and further in view of Callen *et al.,* (US Patent No. 6,492,511 B2, filed on September 7, 1999).

The teachings of Ishikawa *et al.,* Mitsuhashi, Garvin, and Naef have been summarized previously, *supra.*

Ishikawa *et al.,* Mitsuhashi, Garvin and Naef do not disclose that the filter membrane is washed with ethanol as recited in claim 10. However, Mitsuhashi teaches subjecting to vacuum aspiration until the filter membrane is dry as recited in claim 10 (see page 7).

Callen *et al.,* suggest removing most of remaining water on filters using 100% ethanol and allowing the filters to dry (see Example 2 in column 20).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to have performed the method recited in claim 10 wherein the filter membrane is washed with ethanol in view of the prior art of Ishikawa *et al.,* Mitsuhashi, Garvin, Naef, and Callen *et al.*. One having ordinary skill in the art would have been motivated to do so because Callen *et al.,* suggest to remove most of remaining water on filters using 100% ethanol (see Example 2 in column 20). One having ordinary skill in the art at the time the invention was made would have been a reasonable expectation of success to use 100% ethanol in order to remove most of remaining water on filters and allow the filters to dry.

18. Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Ishikawa *et al.,* Mitsuhashi and Garvin as applied to claims 1, 3, 5, 11, 12, 15, and 36-38 above, and further in view of Shinozawa *et al.,* (Leukemia Research, 24, 965-970, 2000).

The teachings of Ishikawa *et al.,* Mitsuhashi and Garvin have been summarized previously, *supra*.

Ishikawa *et al.,* Mitsuhashi and Garvin do not disclose that the specific mRNA is mRNA known to be induced during apoptosis development in leukemia as recited in claim 27.

Shinozawa *et al.,* teach to isolate RNAs from peripheral bloods of leukemia patients and detect survivin mRNA expression wherein survivin mRNA is mRNA known to be induced during apoptosis development in leukemia as recited in claim 27 (see abstract in page 965, pages 965 and 966, and Table 1).

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to have performed the method recited in claim 27 wherein the specific mRNA is mRNA known to be induced during apoptosis development in leukemia such as survivin mRNA in view of the prior art of Ishikawa *et al.,* Mitsuhashi, Garvin, and Shinozawa *et al.*. One having ordinary skill in the art would have been motivated to do so because survivin is a inhibitor of the apoptosis protein and is common in all of the leukemia cell lines (see abstract in page 965, page 967, right column, last paragraph bridging to page 968, left column, first paragraph). One having ordinary skill in the art at the time the invention was made would have been a reasonable expectation of success to perform the method recited in claim 27 by quantifying the specific mRNA such as survivin mRNA.

Conclusion

19. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

20. No claim is allowed.

21. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993)(See 37 CAR § 1.6(d)). The CM Fax Center number is (571)273-8300.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Frank Lu, Ph.D., whose telephone number is (571)272-0746. The examiner can normally be reached on Monday-Friday from 9 A.M. to 5 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla, can be reached on (571)272-0735.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

/Frank W Lu /
Primary Examiner, Art Unit 1634
August 11, 2008